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INHERENTNA BIOLOŠKA RAZGRADNJA VODOOTPORNIH MAZIVA

Sažetak

U slijedu određivanja biološke razgradnje vodootpornih mazivih masti, metodom u kojoj se određuje nastali ugljični dioksid, nastavljena su ispitivanja prema internacionalnom standardu ISO 14593 - CO₂ headspace test i CONCAWE - metoda za procjenu inherentne biološke razgradnje uljnih proizvoda. CONCAWE-metoda "inherentne" biološke razgradnje je modifikacija ISO 14593, koja omogućuje dobivanje informacija o potencijalnoj (inherentnoj) biološkoj razgradnji, inače nerazgradljivih tvari i njihovoj ipak ekološkoj prihvatljivosti. Modifikacija metode ISO 14593 sastoji se u primjeni preeksponiranog cjepiva na ispitivanu tvar i produljenom vremenu trajanja pokusa biološke razgradnje. U ovom su radu predmet ispitivanja mazive masti teško topljive u vodi, na osnovi sintetičkog estera ili biljnog ulja različito ugušćene s litij-sapunom ili alumosilikatom.

Prema dobivenim rezultatima ispitivanja metodom inherentne razgradnje ova inače biološki razgradljiva maziva pokazala su veći postotak razgradnje samo u vremenski produljenom testu.

1. UVOD

Mazive masti su tipične kompleksne smjese ugljikovodika i zgušnjavala, djelomično ili slabo topljive u vodi. Metode određivanja biološke razgradnje moraju biti prilagođene svojstvima maziva.

Najviše upotrebljavana metoda za određivanje biološke razgradnje je CEC-L-33-A-93 [1], koja tijekom pokusa prati samo smanjenje osnovne tvari, tj. primarnu biološku razgradnju. Razgradnja tvari do CO₂, vode i mikrobne

mase određuje se metodama totalne biološke razgradnje koje jedine daju pravu informaciju da u okolišu ne zaostaje sama tvar i njezini razgradni produkti. Jedan od parametara kojim se prati totalna biološka razgradnja je **mjerenje nastalog CO₂** i to metodama:

- Modificirani Sturm-test [2] (preporučena od EU i ISO)
- ISO 14593 - CO₂ headspace test [3]
- CONCAWE – metoda inherentne biološke razgradnje uljnih proizvoda [4]

Princip modificiranog Sturm-testa je da se poznati volumen nacijepljene mineralne podloge koja sadrži odgovarajuću koncentraciju maziva i to 10-20 mg ukupno organski vezanog ugljika TOC/l kao jedini izvor organskog ugljika prozračuje zrakom bez CO₂. Razgradnja se prati tijekom 28 dana određivanjem nastalog CO₂ vezanog na barij hidroksid, titracijom s HCl u dovoljno čestim vremenskim razmacima da se dobije krivulja biološke razgradnje iz koje se može procijeniti brzina i stupanj biološke razgradnje maziva. Stupanj biološke razgradnje u postocima izračuna se iz omjera količine CO₂ nastalog razgradnjom maziva i teoretske količine CO₂.

Princip ISO 14593 - CO₂ headspace metode jest da se ispitivana tvar, kao izvor ugljika (2–40 mg TOC/l) i energije, doda u nacijepljeni mineralni medij u headspace bočice. Biološka razgradnja (mineralizacija do CO₂) prati se mjerenjem porasta anorganskog ugljika tijekom 28 dana i izražava kao postotak u odnosu na teoretsku količinu CO₂. Uvjeti testa su slični kao i kod Sturm-testa. Međutim, ovdje su bočice zatvorene pa je moguće testiranje hlapivih komponenata, a i nema problema s eventualnim gubitkom CO₂.

CONCAWE – metoda inherentne biološke razgradnje uljnih proizvoda kompatibilna je s ISO 14593 osim što se ovdje koristi kultura mikroorganizama prethodno adaptirana na ispitivanu tvar i što test vremenski traje dulje, tj. 56 dana do tri mjeseca.

Svrha ovog rada je primjena metoda ISO 14593 - CO₂ headspace test i CONCAWE - metoda za procjenu inherentne biološke razgradnje uljnih proizvoda na mazive masti i na maziva ulja. Ova su ispitivanja nastavak u nizu ispitivanja istih uzoraka čiji su rezultati prikazani na prethodnim simpozijima MAZIVA i publicirani [5,6,7,8]. Ujedno je prikazana usporedba s rezultatima biološke razgradnje dobivenim modificiranim Sturm-testom.

2. MATERIJALI I METODE

2.1 Uzorci mazivih maziva i mazivih ulja

Ispitivanja biološke razgradnje izvršena su na sljedećim uzorcima:

Uzorak A: Maziva mast na osnovi sintetičkog estera ugušćena alumosilikatom.

Uzorak B: Maziva mast na osnovi biljnog estera ugušćena alumosilikatom.

Uzorak C: Maziva mast na osnovi sintetičkog estera ugušćena litij-kalcij sapunom.

Uzorak D: Maziva mast na osnovi biljnog estera ugušćena litij sapunom.

Uzorak G: Maziva mast na osnovi sintetičkog estera ugušćena litij sapunom.

Uzorak E: Mazivo ulje na osnovi sintetičkog estera.

Uzorak F: Mazivo ulje na osnovi biljnog estera.

Uzorak H: Di-izotridecil adipat (DITA); sintetički ester dikarbonske kiseline; služi kao referentna tvar (RL 130) po metodi CEC L-33-A-93

2.2. Eksperimentalni dio

2.2.1. ISO 14593 - CO₂ headspace test

Za svaki pokus priređena je serija headspace bočica od 160 ml s butilgumenim čepom i aluminijskom kapicom. U njima je mjereno CO₂ nastalo biološkom razgradnjom maziva i referentne tvari (natrij benzoat), zatim CO₂ nastalo endogenom respiracijom bakterija (slijepa proba bez dodatka maziva) te CO₂ u kontroli toksičnosti (mazivo i referentna tvar), kao i u kontroli abiotske razgradnje (mazivo i HgCl₂). Mazivo je vagano direktno u bočice tako da su odvage bile ekvivalentne količini totalnog organskog ugljika, tj. 10 - 15 mg TOC/l. Zatim je dodan mineralni medij i bočice su stavljene u ultrazvučnu kupelj kako bi se postigla što bolja disperzija maziva u mineralnom mediju. Kao cjepivo upotrijebljena je kontinuirano uzgojena kultura iz laboratorijskog uređaja. Porijeklo kulture je filtrat zemlje i aktivni mulj iz uređaja za obradbu otpadnih voda. Nakon toga bočice su hermetički zatvorene i stavljene u inkubiranu tresilicu na temperaturi od 22°C uz trešnju od 150 okretaja/min. Pokus je trajao 28 dana tijekom kojeg su se izuzimale bočice u kojima se mjerio nastali CO₂. Prije mjerenja u svaku je bočicu špricom dodan 1 ml otopine NaOH (c=7 mol/dm³) pa je nastali CO₂ iz plinske faze preveden u tekuću. Nakon jednosatnog ponovnog miješanja na tresilici, mjereno je CO₂ u vodenoj fazi na TOC analizatoru tvrtke Shimadzu.

2.2.2. CONCAWE – metoda “inherentne” biološke razgradnje uljnih proizvoda

Postupak izvođenja ovog testa potpuno je identičan prethodno opisanom postupku pod 2.2.1. ISO 14593 - CO₂ headspace test. Mazivo je vagano direktno u bočice tako da su odvage ekvivalentne količini totalnog organskog ugljika, tj. 10–15 mg TOC/l mineralnog medija. Razlika s prethodno opisanim

testom je u pripremi cjepiva. U kontinuirano uzgojenu mikrobiološku kulturu dodavano je mazivo namijenjeno za ispitivanje sljedećim ritmom: prvi dan dodana je odvaga maziva ekvivalentna s 4 mg TOC/l; sedmi i jedanaesti dan inkubacije dodana je voda izgubljena evaporacijom i odvaga maziva od 8 mg TOC/l; četrnaesti dan preeksponiranja cjepiva, tj. ujedno na dan postavljanja pokusa biološke razgradnje cjepivo je filtrirano i miješano do trenutka korištenja. Pokus biološke razgradnje izvođen je do postignuća "plateau-a".

3. REZULTATI I DISKUSIJA

Rezultati ispitivanja biološke razgradnje mazivih masti i mazivih ulja metodama ISO 14593 - CO₂ headspace test i CONCAWE – metoda inherentne biološke razgradnje uljnih proizvoda prikazani su u tablici 1. Za usporedbu dodani su i rezultati iz prethodnih ispitivanja istih uzoraka samo primjenom Sturm testa. Grafički prikaz biološke razgradnje ispitivanih maziva CONCAWE – metodom inherentne biološke razgradnje uljnih proizvoda prikazan je na slikama 1, 2 i 3.

Tablica 1: Rezultati određivanja stupnja biološke razgradnje u odnosu na teoretsku vrijednost CO₂

Table 1: Results of determining biodegradability level with regard to theoretical CO₂ value

Oznaka uzorka	Stupanj biološke razgradnje (%) CO ₂ /TCO ₂			
	Modificirani Sturm-test	ISO 14593 - CO ₂ headspace test	CONCAWE – metoda inherentne biološke razgradnje uljnih proizvoda	
	28. dan	28. dan	28.dan	43.* (odn. 56.) dan
Uzorak A	84,1	87,3	87,7	88,0*
Uzorak B	77,3	84,4	89,2	92,0*
Uzorak C	61,2	67,7	62,7	71,6
Uzorak D	68,0	77,3	87,9	90,3
Uzorak G	57,9	71,7	74,3	75,0
Uzorak E	30,0	40,3	37,9	37,7
Uzorak F	86,8	85,1	86,6	92,8
Uzorak H(DITA)	41,0	49,1	67,4	68,7
Referentna tvar Na-benzoat	91,8	92,4	89,5	89,6

Sample Mark/Biodegradability Level/Modified Sturm-test/ 28th day/ ISO 14593 - CO₂ headspace test/ CONCAWE method of inherent biodegradability of oil products/ 43rd (i.e. 56th) day/Sample (.) /Referential Substance Na-benzoate

Uzorci mazivih masti A i B pokazali su visoki stupanj biološke razgradnje i to naročito uzorak B (92,0%) koji je na osnovi biljnog estera. Također su oba maziva "brzo" biološki razgradljiva. To je uočeno analiziranjem rezultata

10-dnevnog razdoblja, gdje je CONCAWE – metodom inherentne biološke razgradnje uljnih proizvoda dvanaesti dan pokusa mazivo A dostiglo 61%, a mazivo B 63% razgradnje. Kod ISO 14593 - CO₂ headspace testa ovaj je 10-dnevni prozor postignut već 11. dan pokusa. Uspoređujući konačne rezultate između biološke razgradnje uzoraka mazivih masti A i B, dobivenih metodama ISO 14593 - CO₂ headspace test i CONCAWE – metoda inherentne biološke razgradnje uljnih proizvoda, 28. dan pokusa i 43. dan kada je postignut “plateau”, nema značajnih razlika.

Kod uzoraka C i G, koji su na osnovi sintetičkog estera, nema značajnih razlika u rezultatima dobivenim objema metodama (ISO 14593 - CO₂ headspace test i CONCAWE – metoda inherentne biološke razgradnje uljnih proizvoda) i 28. dan pokusa i 56. dan kada je postignut “plateau”. Značajna razlika u dobivenim rezultatima između obiju metoda uočena je kod uzorka D, na osnovi biljnog estera i to za 10% 28. dan pokusa, a za vremenski produljeni test 13%. On je također “brzo” biološki razgradljiv jer je 10-dnevni prozor postignut već 12. dan pokusa.

Maziva ulja (uzorci E i F) već su po svojoj konzistenciji različita od ispitivanih maziva i drukčije se ponašaju u vodi. Uzorak E je mazivo ulje na osnovi sintetičkog estera i niti po jednoj metodi biološke razgradnje nije biološki razgradljiv. Uzorak F je mazivo ulje na osnovi biljnog estera. Ovo je mazivo ulje postiglo visoki stupanj biološke razgradnje od 92,8 % u vremenski produljenom testu i uz adaptiranu mikrobiološku kulturu. Po objema ispitivanim metodama (ISO 14593 - CO₂ headspace test i CONCAWE – metoda inherentne biološke razgradnje uljnih proizvoda) ovo je mazivo ulje 28. dan pokusa postiglo biološku razgradnju od 85,1% i 86,6% u odnosu na teorijsku vrijednost. Također je brzo biološki razgradljivo jer je “10-dnevni prozor” postignut već 11. dan pokusa.

Di-izotridecil adipat (DITA) uključen je u ova analitička ispitivanja [5] kada je Sturm testom dokazana biološka razgradnja od 41%. Metodom ISO 14593 - CO₂ headspace test postignut je 28. dan pokusa 49,1% razgradnje, a CONCAWE – metodom inherentne biološke razgradnje uljnih proizvoda 56. dan pokusa 68,7%. Prema tome bi se taj ester dikarbonske kiseline ipak mogao ocijeniti kao biološki razgradljiv, ali tek uz adaptiranu mikrobiološku kulturu i vremenski produljeni test. Do sličnih su spoznaja došli i drugi autori [9].

Tablica 2: Valjanost pokusa za mazive masti prema ISO 14593-CO₂ headspace testuTable 2: Validity of the test for lubricating greases according to the ISO 14593 - CO₂ headspace test

	Kriteriji metode	Postignute vrijednosti (%)				
		A	B	C	D	G
Količina nastalog anorganskog ugljika u kontrolnom pokusu (slijepa proba)	≤15% od količine dodanog TOC	9,2	10,1	8,9	8,8	8,9
Razlike vrijednosti stupnja razgradnje u usporednom pokusu (28. dan)	< 20 %	3,0	7,9	7,6	3,8	5,4
Stupanj razgradnje referentne tvari (14. dan)	≥ 60 %	87,5	87,5	87,5	87,5	87,5
Stupanj razgradnje u pokusu toksičnosti (14. dan)	≥ 25 %	65	76	69	71	81

The Method's Criteria/The Values Achieved/Volume of the inorganic carbon generated in the control test (blind test): ≤ 15% of the added TOC volume/

Differences in the degradation level value in the comparative test (28th day)/Referential substance degradation level (14th day)/Degradation level in toxicity test (14th day)

Tablica 3: Valjanost pokusa za mazive masti prema CONCAWE™-metodi inherentne biološke razgradnje uljnih proizvoda

Table 3: Validity of the test for lubricant greases according to the CONCAWE method of inherent biodegradability of oil products

	Kriteriji metode	Postignute vrijednosti (%)				
		A	B	C	D	G
Količina nastalog anorganskog ugljika u kontrolnom pokusu (slijepa proba)	≤15% od količine dodanog TOC	15,0	14,3	14,9	14,5	9,2
Razlike vrijednosti stupnja razgradnje u usporednom pokusu (28. dan)	<20 %	3,0	13,8	7,2	10,7	9,7
Stupanj razgradnje referentne tvari (14. dan)	≥60 %	87,8	87,8	87,8	87,8	87,1
Stupanj razgradnje u pokusu toksičnosti (14. dan)	≥25 %	60,4	73,6	65,1	87,9	87,5

The Method's Criteria/ The Values Achieved/Volume of the inorganic carbon generated in the control test (blind test): ≤ 15% of the added TOC volume/Differences in the degradation level value in the comparative test (28th day)/Referential

substance degradation level (14th day)/Degradation level in toxicity test (14th day)

Tablica 4: Valjanost pokusa za maziva ulja i DITA prema ISO 14593-CO₂ headspace testu

Table 4: Validity of the test for lubricant oils and DITA according to the ISO 14593 - CO₂ headspace test

	Kriteriji metode	Postignute vrijednosti (%)		
		E	F	DITA
Količina nastalog anorganskog ugljika u kontrolnom pokusu (slijepa proba)	≤15% od količ. dodanog TOC	11,1	11,8	11,8
Razlike vrijednosti stupnja razgradnje u usporednom pokusu (28. dan)	<20 %	6,1	0,8	18,9
Stupanj razgradnje referentne tvari (14. dan)	≥60 %	95,3	95,3	95,3
Stupanj razgradnje u pokusu toksičnosti (14. dan)	≥25 %	72,8	87,1	77,9

The Method's Criteria/The Values Achieved/Volume of the inorganic carbon generated in the control test (blind test): ≤ 15% of the added TOC volume/Differences in the degradation level value in the comparative test (28th day)/Referential substance degradation level (14th day)/Degradation level in toxicity test (14th day)

Tablica 5: Valjanost pokusa za maziva ulja i DITA prema CONCAWE-metodi inherentne biološke razgradnje uljnih proizvoda

Table 5: Validity of the test for lubricant oils and DITA according to the CONCAWE method of inherent biodegradability of oil products

	Kriteriji metode	Postignute vrijednosti (%)		
		E	F	DITA
Količina nastalog anorganskog ugljika u kontrolnom pokusu (slijepa proba)	≤ 15% od količ. dodanog TOC	9,6	9,9	9,7
Razlike vrijednosti stupnja razgradnje u usporednom pokusu (28. dan)	< 20 %	9,6	9,7	6,8
Stupanj razgradnje referentne tvari (14. dan)	≥ 60 %	87,1	87,1	87,1
Stupanj razgradnje u pokusu toksičnosti (14. dan)	≥ 25 %	82,5	87,5	72

The Method's Criteria/The Values Achieved/Volume of the inorganic carbon generated in the control test (blind test): ≤ 15% of the added TOC volume/Differences in the degradation level value in the comparative test (28th day)/Referential substance degradation level (14th day)/Degradation level in toxicity test (14th day)

Radi bolje usporedbe rezultata i u pokusu "inherentne" biološke razgradnje kao referentna tvar korišten je natrij benzoat, premda CONCAWE metoda "inherentne" biološke razgradnje uljnih proizvoda preporuča n-heksadekan. Natrij benzoat se u svim izvedenim pokusima podjednako razgradio što ukazuje da je upotrijebljena mikrobiološka kultura bila dobre aktivnosti u

svim pokusima. U tablicama 2 do 5 prikazani rezultati potvrđuju valjanost pokusa, odnosno zadovoljavanje uvjeta vođenja pokusa propisanih određenim metodama. Iz dobivenih rezultata utvrđeno je, između ostalog, da niti jedan uzorak nije toksično djelovao na mikrobiološku kulturu (14. dan pokusa nastali CO₂ u odnosu na teorijsku vrijednost CO₂ bio je u rasponu od 60 - 87%).

4. ZAKLJUČAK

Mnogi uljni proizvodi nisu brzo biološki razgradljivi pa su podaci o njihovoj potencijalnoj inherentnoj biološkoj razgradnji vrlo korisni kod procjene njihovog utjecaja na okoliš.

CONCAWE – metoda inherentne biološke razgradnje uljnih proizvoda istovjetna je s ISO 14593 - CO₂ headspace testom samo što CONCAWE metoda koristi preekspanirano cjepivo na ispitivanu tvar, a trajanje pokusa može biti vremenski produljeno dok se ne postigne plateau. CONCAWE metoda je u ovom radu korištena za određivanje biološke razgradnje mazivih masti na osnovi sintetičkih i biljnih estera različito ugušćenih. Samo kao paralelna usporedba, pod istim uvjetima izvođena je i biološka razgradnja mazivih ulja (uzorci E i F), te ester dikarbonske kiseline (DITA).

Preekspanirano cjepivo na ispitivana maziva i produljeno vrijeme testiranja primjenom CONCAWE – metode inherentne biološke razgradnje uljnih proizvoda omogućili su dobivanje različito povišenih rezultata biološke razgradnje u odnosu na rezultate dobivene Sturm testom i ISO 14593 - CO₂ headspace testom:

- kod mazivih masti na osnovi sintetičkih estera to povišenje rezultata u odnosu na ISO 14593 - CO₂ headspace test iznosi svega 1–4%, a u odnosu na Sturm test 4–17%;
- kod mazivih masti na osnovi biljnog estera povišenje rezultata u odnosu na ISO 14593 - CO₂ headspace test iznosi 8–13%, a u odnosu na Sturm test 12–22%;
- kod mazivih ulja na osnovi biljnih estera u odnosu na obje metode povišenje rezultata iznosi 6%;
- značajan rezultat dobiven je za di-izotridecil adipat (DITA), koji je po Sturm testu 28. dan pokusa ocijenjen kao biološki nerazgradljiv (41%), ali uz preekspaniranu mikrobiološku kulturu i 56 dana razgradnje, postignuta vrijednost biološke razgradnje u odnosu na teoretsku iznosila je više od 60% (68,7%) pa se ipak može ocijeniti kao potencijalno biološki razgradljiv.

U tijeku su ispitivanja toksičnog djelovanja spomenutih maziva na alge.

Slika 1: Biološka razgradnja mazive masti A

Figure 1: Biodegradability of lubricating grease A

Slika 2: Biološka razgradnja mazive masti B

Figure 2: Biodegradability of lubricating grease B

Slika 3: Biološka razgradnja mazive masti C

Figure 3: Biodegradability of lubricating grease C

Slika 4: Biološka razgradnja mazive masti D

Figure 4: Biodegradability of lubricating grease D

Slika 5: Biološka razgradnja mazive masti G

Figure 5: Biodegradability of lubricating grease G

Slika 6: Biološka razgradnja mazivog ulja E

Figure 6: Biodegradability of lubricating oil E

Slika 7: Biološka razgradnja mazivog ulja F

Figure 7: Biodegradability of lubricating oil F

Slika 8: Biološka razgradnja uzorka Di-izotridecil adipata (DITA)

Figure 8: Biodegradability of Diisotridecyl adipate (DITA)

INHERENT BIODEGRADABILITY OF WATER-RESISTANT GREASES

Abstract

In the process of establishing the biodegradability of water resistant greases by using the method applied to assess the resulting carbon dioxide, the analysis were proceeded according to the international norm ISO 14593 - CO₂ headspace test and the CONCAWE test - method to assess the inherent biodegradability of oil products. The CONCAWE method of inherent biodegradability is a modified ISO 14593, which is used to obtain information on potential (inherent) biodegradability of otherwise non degradable substances and hence of their potential ecological acceptability. The modified ISO 14593 method consists in the pre-exposed inoculum applied to the test substance and the extended time of biodegradability test. This paper deals with an analysis of water insoluble greases on synthetic ester or vegetable oil basis, thickened either with a lithium soap or aluminium silicate.

Based on the results achieved by inherent biodegradability method, these otherwise biodegradable greases demonstrated a higher percentage of degradability only during an extended time test.

1 INTRODUCTION

Greases are typical complex hydrocarbon compounds, partially or poorly water-soluble. The methods of determining biodegradability must be adapted to the properties of the greases.

The method used the most for biodegradability determination is CEC-L-33-1-93 /1/, which, during the experiment, monitors only the basic substance reduction i.e. primary biodegradability. Degradation of substances down to CO₂, water, and microbic mass is determined by the methods of total biodegradability which are the only ones to provide the real information that the substance itself or its degradation products are not left over in the environment. One of the parameters monitoring total biodegradability is **the measuring of the generated CO₂**, in the following methods:

- Modified Sturm-test /2/ (recommended by EU and ISO)
- ISO 14593 – CO₂ headspace test /3/
- The CONCAWE method of inherent biodegradability of oil products /4/.

The principle of the modified Sturm-test is that the known volume of inoculated mineral basis containing a given grease concentration of 10-20 mg total organic carbon TOC/l as the only organic carbon source is ventilated by CO₂ free air. Degradation is monitored for 28 days by determining generated CO₂ bonded with barium hydroxide, through titration with HCl in sufficiently frequent intervals as to obtain a biodegradability curve allowing the estimation of the speed and level of grease biodegradability. Biodegradability level (%) is calculated on the basis of the ratio between CO₂ generated through grease degradation and theoretical CO₂ volume.

The principle of the ISO 14593 – CO₂ headspace method is that the tested substance as the source of carbon (2-40 mg TOC/l) and energy is added into the inoculated mineral medium in headspace bottles. Biodegradability as mineralization down to CO₂ is monitored by measuring the increase of inorganic carbon during 28 days and is expressed as percentage with regard to theoretical CO₂ volumes. Test conditions are similar as in the case of the Sturm-test. However, in this case the bottles are sealed, thus permitting the testing of volatile components, while there are no problems with possible CO₂ losses either.

The CONCAWE Method of Inherent Biodegradability of Oil Products is compatible with ISO 14593, only, in this case, the microorganism culture previously adapted to the tested substance is used, and the duration extended: From 56 days to three months. The purpose of the present paper is the application of the ISO 14593 - CO₂ headspace test and the CONCAWE method for estimating the inherent biodegradability of oil products on lubricating greases and lubricating oils. These tests are a continuation of testing the same samples, the results of which were shown on previous LUBRICANTS Symposia and published /5,6,7,8/. There is also the comparison with biodegradability results obtained through modified Sturm-test.

2 MATERIALS AND METHODS

2.1 Lubricating Greases and Lubricating Oils Samples

Biodegradability tests were performed on the following samples:

Sample A: Lubricating grease based on synthetic ester, thickened with aluminium silicate.

Sample B: Lubricating grease based on vegetable ester thickened with aluminium silicate.

Sample C: Lubricating grease based on synthetic ester, thickened with lithium/calcium soap.

Sample D: Lubricating grease based on vegetable ester thickened with lithium soap.

Sample G: Lubricating grease based on synthetic ester, thickened with lithium soap.

Sample E: Lubricating oil based on synthetic ester.

Sample F: Lubricating oil based on vegetable ester.

Sample H: DITA: synthetic ester of dicarbonic acid, - serves as referential substance (RL 130), according to the method CEC L-33-A-93

2.2 The Experimental Part

2.2.1 ISO 14593 – The CO₂ Headspace Test

For every test, a series of headspace bottles has been prepared: of 160 ml and with butyl rubber stopper and aluminum cap. The following measurements were performed in them: CO₂ generated by biodegradability of the grease and of the referential substance (sodium benzoate); CO₂ generated by endogenous respiration of bacteria (blind test without grease addition), and CO₂ in toxicity control (grease and referential substance, as well as in the control of abiotic degradation (grease and HgCl₂). The grease was weighed directly into bottles, so that the measured volumes were equivalent to the volume of total organic carbon i.e. 10-15 mg TOC/l. After that, the mineral medium was added, and the bottles put into an ultrasonic bath in order to achieve as good a dispersion of the grease in the mineral medium as possible. As inoculum, we used a continuously grown culture from the laboratory device. The origin of the culture is soil filtrate and active sludge from the wastewater treatment device. After that, the bottles were sealed and placed into an incubated shaker at the temperature of 22°C, with shaking of 150 rpm. The test lasted 28 days during which the bottles in which the generated CO₂ was measured were being taken out. Before the measurement, each bottle was added an injection of 1 ml of NaOH solution ($c=7 \text{ mol/dm}^3$), and so the generated CO₂ was transformed from the gaseous phase to the liquid one. After a repeated one-hour mixture on the shaker, the CO₂ in aqueous phase was measured on a TOC analyzer manufactured by the Shimadzu company.

2.2.2 The CONCAWE Method Of inherent Biodegradability Of Oil Products

The manner of performing this test is quite identical to the previously described procedure under 2.2.1: ISO 14593 – The CO₂ Headspace Test.

The grease was weighed directly into bottles, so that the measured volumes were equivalent to the volume of total organic carbon i.e. 10-15 mg of TOC/l of the mineral medium. The difference with regard to the previously described test consists in inoculum preparation. A continuously grown microbiological culture was added grease intended for testing according to the following rhythm: the first day the volume of grease added was equivalent to 4 mg TOC/l; on the 7th and 11th incubation day water was added that had been lost through evaporation and grease volume in the amount of 8 mg TOC/l; on the 14th day of the inoculum's pre-exposition i.e. on the day when the biodegradability test was set, the inoculum was filtered and stirred until the moment of use. The biodegradability test was performed until "plateau" has been achieved.

3. RESULTS AND DISCUSSION

The results of testing the biodegradability of lubricant greases and lubricant oils using the ISO 14593 - CO₂ headspace test and CONCAWE method of inherent biodegradability of oil products are shown in Table 1. For the sake of comparison, results have been added from the previous tests of the same samples using the Sturm Test. The graphical biodegradability presentation of the greases tested using the CONCAWE method of inherent biodegradability of oil products is shown in Figures 1, 2 and 3.

A and B lubricating grease samples have shown a high biodegradability level, especially Sample B (92.0%), based on vegetable ester. Both greases are at the same time readily biodegradable. This has been observed by analyzing the results of the 10-day period, where, using the CONCAWE method of inherent biodegradability of oil products, on the 12th day of the test, grease A reached 61% and grease B 63% of degradation. As regards the ISO 14593 - CO₂ headspace test, the said 10-day window was reached already on the 11th day of the test.

By comparing the final results between biodegradability of A and B lubricant grease samples obtained using the ISO 14593 - CO₂ headspace test and CONCAWE method of inherent biodegradability of oil products, on both 28th and 43rd day of the test, when plateau has been reached, it turned out that there were no major differences.

As regards C and G samples, based on synthetic ester, there were no major differences between the results obtained by both methods (ISO 14593 - CO₂ headspace test and CONCAWE method of inherent biodegradability of oil products), on both 28th and 56th day of the test, when the plateau has been reached. A major difference between the results obtained by these methods has been observed for Sample D, based on vegetable ester: By 10% on the 28th day of the test, and by 13% for the test of prolonged duration. It is also readily biodegradable, since the 10-day window was reached already on the 12th day of the test.

Lubricating oils (Samples E and F) are by their very consistency different from lubricants tested and behave differently in water. Sample E is a lubricating oil based on synthetic ester and is not biodegradable under any of biodegradability methods.

Sample F is a lubricating oil based on vegetable ester. This lubricating oil has reached a high biodegradability level of 92.8% in the test of prolonged duration, with an adapted microbiological culture. According to both test methods (ISO 14593 - CO₂ headspace test and CONCAWE method of inherent biodegradability of oil products), on the 28th day, the lubricant oil reached biodegradability in the amount of 85.1 and 86.6% respectively with regard to the theoretical value. It is also readily biodegradable, since the 10-day window was reached already on the 11th day of the test.

DITA was included into these analytical investigations /5/ when biodegradability in the amount of 41% was proven using the Sturm Test. Using the ISO 14593 - CO₂ headspace test, 49.1% of degradation was achieved on the 28th day of the test, while, using the CONCAWE method of inherent biodegradability of oil products, a 68.7% degradation was achieved on the 56th day of the test. According to this, the said dicarbonic acid ester could still be considered biodegradable, but only with an adapted microbiological culture and the test of prolonged duration. Other authors have come up with similar results as well /9/.

For the sake of a better comparison of results, in the inherent biodegradability test, sodium benzoate was used as referential substance as well, although the CONCAWE method of inherent biodegradability of oil products recommends n-hexadecane. Sodium benzoate was similarly degraded in all of the tests performed, pointing that the microbiological culture used was of good activity in all tests. In Tables 2 - 5, the results shown confirm the validity of the tests i.e. the meeting of test conducting conditions set by

specific methods. The results obtained show, among other things, that not a single sample had a toxic impact on the microbiological culture (CO₂ generated on the 14th day of the test ranged from 60-87% with regard to the theoretical CO₂ value).

4. CONCLUSION

Many oil products are not readily biodegradable, which is why the data on their potential inherent biodegradability are most useful when assessing their environmental impact.

The CONCAWE Method of inherent Biodegradability of Oil Products is identical with the ISO 14593 - CO₂ headspace test, only, the former uses pre-exposed inoculum on the tested substance, while the test duration may be extended until plateau is achieved. The CONCAWE Method has been used in this paper for determining the biodegradability of lubricating greases based on both synthetic and vegetable esters, thickened in different ways. Just as a parallel comparison, biodegradability of lubricating oils (samples E and F) and dicarbonic acid ester (DITA) was conducted under the same conditions.

The pre-exposed inoculum on the greases tested and prolonged test duration using the CONCAWE Method of inherent Biodegradability of Oil Products have enabled the yielding of differently increased biodegradability results with regard to results obtained by the Sturm Test and the ISO 14593 - CO₂ headspace test:

- in the case of lubricating greases based on synthetic esters, this results increase amounts to only 1-4% with regard to the ISO 14593 - CO₂ headspace test, and to 4-17% with regard to the Sturm Test,
- in the case of lubricating greases based on vegetable ester, the increase of results amounts to 8-13% with regard to the ISO 14593 - CO₂ headspace test, and to 12-22% with regard to the Sturm Test;
- in the case of lubricating oils based on vegetable esters, the results increase amounts to 6% in the case of both methods;
- a significant result has been obtained for DITA: According to the Sturm Test, it was evaluated as biologically non-degradable on the 28th day of the test (41%), but, with the pre-exposed microbiological culture and 56 days of degradation, the biodegradability level achieved with regard to that theoretical amounted to over 60% (68.7%), which is why it may still be evaluated as potentially biodegradable.

Tests of toxic impact of the said greases on algae are currently under way.

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